Patent
Attorney's Docket No. 030560-056

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of	)	**MAILSTOP AF**
Andreas BERNKOP-SCHNÜRCH	)	Group Art Unit: 1619
Application No.: 09/830,986	)	Examiner: Shahnam J. Sharareh
Filed: April 20, 2001	)	Confirmation No.: 7285
For: MUCO-ADHESIVE POLYMERS, USE THEREOF AND METHOD FOR PRODUCING THE SAME	)	

#16
AKO
8-21-03

SECOND DECLARATION UNDER 37 C.F.R. §1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Andreas Bernkop-Schnürch, Ph.D., hereby declare as follows:

1. I am the named inventor for the above-identified application.

2. My Curriculum Vitae was attached as Appendix A to my First Declaration previously submitted for this application.

3. I am a person of at least ordinary skill in the art of mucoadhesives.

4. I have read the instant application as well as the Official Action dated April 9, 2003, and Constancis et al, U.S. Patent No. 5,496,872, which is cited therein.

5. I do not agree with the assertions in the Official Action that the instantly claimed invention is disclosed by or obvious in view of Constancis et al, U.S. Patent No. 5,496,872, or is obvious in view of Bernkop-Schnürch in combination with Constancis.

6. The instant invention relates to mucoadhesive polymers having improved properties. The improved mucoadhesive polymers "enable a targeted introduction of active substance in mucus layers, wherein a stable presence at the target site shall be enabled." By this invention, an effective and efficient active substance delivery system is provided "by which an improved and thus also extended adhesion of drug on the mucosae can be attained." Page 2, ¶2, of the application.

7. The term "mucoadhesive" is recognized in the art. It is a term of art that is used to describe a particular class of polymers. U.S. Patent No. 5,047,244, for example, defines "mucoadhesive"as being "a material that adheres to a mucosal tissue surface in-vivo and/or in-vitro. Such adhesion will adherently localize the dosage form onto the mucus membrane and requires the application of a force of at least about 50 dynes/cm^2 to separate the mucoadhesive material from the mucus membrane." Col. 3, lns. 21-27.

8. The term "mucoadhesive," as used herein, is a polymer that adheres to the mucus layer covering a mucosal tissue surface in-vivo and/or in-vitro. Such adhesion has to be higher than at least 83 μJ for the total work of adhesion (TWA) described for tensile studies with dry compacts, according to Bernkop-Schnürch et al. *Pharm. Res.* 16, 1999, 876-881.

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9. Mucoadhesive polymers are recognized in the art as including polyacrylates, (e.g., carbomer, polycarbophil, carbopol, etc.), cellulose derivatives (e.g., sodium carboxymethylcellulose, hydroxypropylcellulose, etc.), hyaluronic acid, alginate, pectin and chitosan. *See, e.g.,* references cited in my First Declaration, including Bernkop-Schnürch, A. (2002). *Mucoadhesive Polymers In: Polymeric Biomaterials* 2nd edition (Ed. Severian

Dumitriu) Marcel Dekker, New York.

10. Constancis does not disclose or suggest "mucoadhesive" polymers, as instantly claimed. Constancis instead relates to "biocompatible and biodegradable surgical adhesives based on non-toxic products." Col. 1, lns. 39-42. More specifically, Constancis discloses biological "glues or gluing material." Col. 5, lns. 24-25. It is obvious that nobody would use a surgical adhesive to try to connect one mucus gel layer with another, or to try to connect a mucus gel layer with a tissue.

11. All experiments described herein were done either by me or under my direct supervision or control.

12. The bioadhesives of Constancis are not "mucoadhesives." This would be apparent to a person skilled in the art.

13. In order to substantiate this assertion, all polymers mentioned in Constancis, U.S. Patent 5,496,872, for which the synthesis is described therein, were synthesized and, where described in U.S. Patent 5,496,872, their chemical structure was confirmed by ^{1}H-NMR, IR and MALDI-TOF MS spectral analyses. The specific description of the Experimental method employed is as follows. The Appendix to my Declaration includes the spectral analyses for the prepared polymers.

14. In the case of the crosslinked Polymer 2, the one crosslinked by cystine dimethyl ester (example 6 and 9) and not the one crosslinked by cystine diethyl ester (example 7 and 10) was chosen as the more hydrophilic polymer, *i.e.*, the one with the methyl esters has a slightly higher chance of being mucoadhesive.

15. Cystine dimethylester dihydrochloride and succinyl chloride (95%) were purchased from Acros Organics (Geel, Belgium). Dimethylacetamide (DMAC), dithiothreitol (DTT) and N'-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC) were purchased from Aldrich (Steinheim, Germany). Triethylamine (TEA) was purchased from Merck (Darmstadt, Germany). NMR-solvents deuteriumoxide (D_2O) and dimethylsulfoxide-d_6 (DMSO-d_6) were purchased from Aldrich and trifluoroacetic acid-d (TFA-d) from Acros Organics. Organic solvents and succinyl chloride were distilled prior to use.

16. ^{1}H-NMR-spectra were recorded on a Bruker Avance DPX 200 NMR-spectrometer at 200 MHZ. Chemical shifts are denoted in δ units (ppm) relative to the according solvent signals .

17. IR-spectra were recorded on a Perkin-Elmer 1000 FT-IR spectrometer using KBr (Merck, Germany) discs.

18. MALDI-TOF-mass spectra were recorded on a Kratos Kompact SEQ spectrometer using 1,8,9-trihydroxyanthracene (Aldrich, Germany) as matrix and a 0.1 M solution of Na-trifluoroacetate in THF as cation donor according to Kratos manual *Analysis of Polymers using higher MALDI-TOF MS* (Resch M., Pasch H,. Ghahary R.). Experiments were run in positive reflectron mode at 100% laser power.

19. Samples were lyophilized in a Christ Beta 1-8K freeze dryer.

20. **Synthesis of the polymer (1) by solution polycondensation in dimethylacetamide (DMAC) of cystine dimethyl ester hydrochloride and succinyl chloride (according to example 1 of Constancis)**

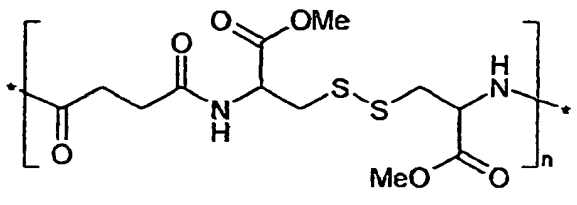


Fig. 1: Subunit of polymer (1).

20.1. 1.8 g of cystine dimethylester dihydrochloride (5.26 mmols) were placed in a 100 ml round-bottom flask and suspended in 30 ml of DMAC. 3 ml of TEA were added. 0.58 ml of succinyl chloride were diluted in 5 ml DMAC and were added via dropping funnel to the suspension. This had to be done rather quickly in order to avoid the occurrence of a dark red colour, which led to intensely coloured products. The mixture was stirred for 24 hours at room temperature. The precipitated triethylammonium salt was removed by suction filtration and the filtrate was precipitated in 800 ml of H_2O. The polymer was recovered by suction filtration and oven-dried under vacuum. 850 mg of a slightly beige/pink – coloured product was obtained.

20.2. The chemical structure of the resulting polymer (1) according to the chemical structure depicted in example 1 was confirmed by ^{1}H-NMR-, IR-, and MALDI-TOF MS spectral analyses (spectral data see appendix).

20.2.1. ^{1}H-NMR (DMSO-d_6/TFA-d) (due to the fact that the spectrometer at our disposal did not provide the lock parameters for TFA-d, the solvent used in US 5,496,872, the sample was dissolved in one drop of TFA-d and was diluted with DMSO-d_6. Chemical shifts

are relative to $\delta_{DMSO\text{-}d6}$ at 2.50 ppm):

δ (ppm): 4.58-4.51 (2H, m, 2 α-H), 3.63 (6H, s, 2 $COOCH_3$), 3.15-2.78 (4H, m, aliphat. H), 2.38 (4H, s, aliphat. H)

20.2.2. IR: (cm^{-1}) 1733 (ester), 1640 (amide I), 1539 (amide II), 1204 (ester)

20.2.3. MALDI-TOF MS: The molecular weight of the resulting polymer was determined via MALDI-TOF mass spectral analysis. The recurring pattern of the subunits (M_r = 350, Fig. 1) can easily be seen.

21. **Hydrolysis of the ester functions of the polymer (1): production of polymer (2) (according to example 3)**

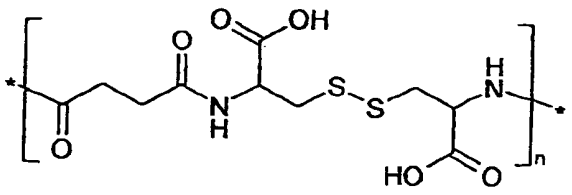


Fig. 2: Subunit of polymer (2)

21.1. 650 mg of polymer (1) were suspended in 130 ml of H_2O. According to the authors of US 5,496,872 the pH should now be adjusted to 10.5 with 1 M NaOH. At this pH we could not observe hydrolysis, indicated by a clear solution. Further addition of 1 M NaOH till a pH of 13 was reached, resulted in the desired saponification (clear solution) within approximately 3 hours.

21.2. Dowex 50 W X 8 as a cationic ion exchange resin was added to adjust the pH to

3. The solution was first concentrated on a rotary evaporator, then frozen and freeze dried. 412 mg of a white powder were obtained.

21.3. The chemical structure of the resulting polymer (2) according to the chemical structure depicted in example 3 was confirmed by ^{1}H-NMR- and IR-spectral analyses (*see*, appendix for spectral data).

21.3.1. ^{1}H-NMR (D_2O) (chemical shifts are relative to δ_{HOD} at 4.8 ppm): δ (ppm): 4.69-4.51 (2H, m, 2 α-H), 3.58-2.49 (8H, m, aliphat. H)

21.3.2. IR: (cm^{-1}) 1636 (amide I), 1591, 1536 (amide II), 1392; the ester moiety cannot be detected.

22. **Reduction of the polymer (2) by dithiothreitol: production of the molecule (3) (according to example 4)**

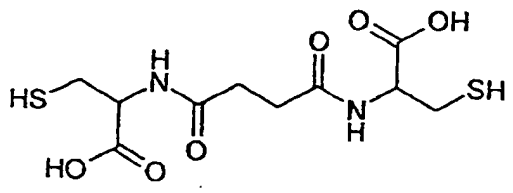


Fig. 3: Reduced dithiol (3)

22.1. 120 mg of polymer (2) and 110 mg of dithiothreitol (DTT) were dissolved in 5 ml of H_2O under a nitrogen atmosphere. The pH was adjusted to 8.5 by addition of 1 M NaOH and the solution was stirred for 3 hours under bubbling with nitrogen. The reaction mixture was then extracted twice with 8 ml of ethyl acetate. The aqueous phase was acidified to pH 4.5 with Dowex 50 W X 8, concentrated and precipitated in acetone. The obtained

precipitate was re-dissolved in H_2O and re-precipitated in acetone. It was filtered, re-dissolved in H_2O, frozen and freeze-dried according to example 4. 106 mg of an almost white product was obtained.

22.2. The chemical structure of the resulting molecule (3) according to the chemical structure depicted in example 4 was confirmed by ^{1}H-NMR spectral analysis (*see*, appendix for spectral data).

22.2.1. ^{1}H-NMR (D_2O) (chemical shifts are relative to δ_{HOD} at 4.8 ppm): δ (ppm): 4.51 (2H, t, J = 5.4 Hz, 2 α-H), 2.95 (4H, d, J = 5.6 Hz, 2 HS-C$\underline{H_2}$-CH), 2.76-2.59 (4H, m, succinyl-H)

22.3. The presence of free thiol groups was verified using 5,5'-dithiobis(2-nitrobenzoic acid) (*Ellman's* reagent, Sigma, Germany) in buffered aqueous medium (0.5 M phosphate buffer, pH = 8), as a deep yellow colour occurred.

23. **Crosslinking of the polymer (2) by cystine dimethyl ester (according to example 6):**

23.1. 130 mg of saponified polymer (2) and 140 mg of cystine dimethyl ester dihydrochloride were dissolved in 4 ml of H_2O. 160 mg of N'-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC) in 1 ml of H_2O were added. The solution turned red immediately and a precipitate formed as described.

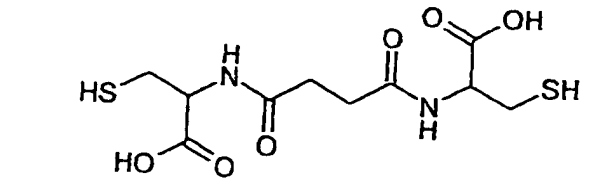
23.2. After 3 hours 5 ml of H_2O were added and the suspension was suction filtrated and washed with water. Finally, the obtained polymer was dried in vacuum (yield: 100 mg).

24. **Reduction by dithiothreitol of the crosslinked polymer (according to example 8):**

24.1. 200 mg of crosslinked polymer were suspended in 10 ml of H_2O, which was bubbled with a stream of N_2. 200 mg of dithiothreitol (DTT) were added and the pH was adjusted to 9.5 by addition of 1 M NaOH. The suspension became clear and after one hour the reaction was stopped.

24.2. The solution was extracted 6 times with ethyl acetate and the aqueous phase was acidified to pH 5 with Dowex 50 W X 8. It was re-extracted with ethyl acetate then frozen and freeze-dried.

24.3. 80 mg of a slightly yellow powder comprising molecules (3), (4) and (5) were obtained. The presence of free thiol groups was verified using 5,5'-dithiobis(2-nitrobenzoic acid) (*Ellman's* reagent, Sigma, Germany) in buffered aqueous medium (0.5 M phosphate buffer, pH = 8), as a deep yellow colour occurred.



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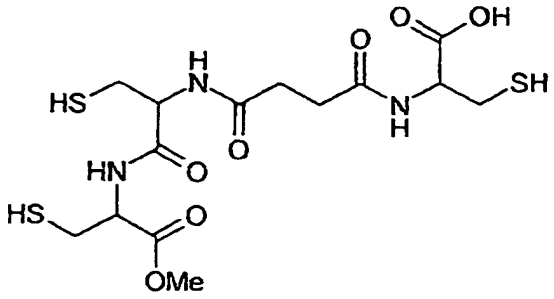

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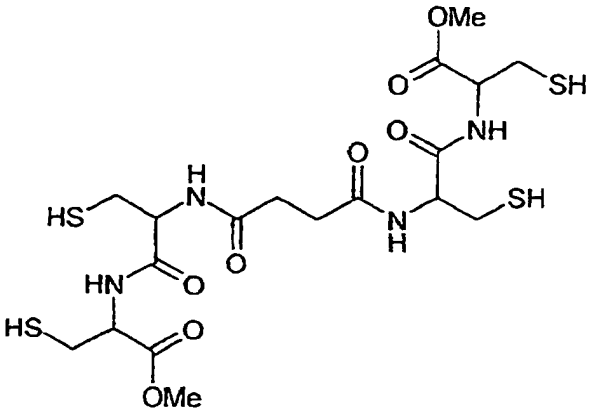


(5)

Fig. 4: Obtained molecules (3), (4) and (5) after reduction with DTT

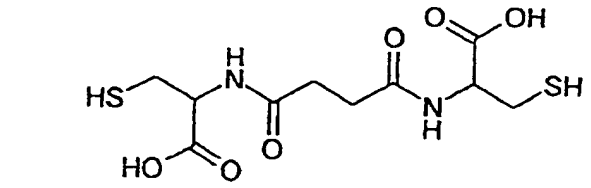
25. **Reduction by dithiothreitol of the crosslinked polymer of example 6 - hydrolysis of the ester functions of the product obtained (according to example 9):**

25.1. The reaction was performed using 100 mg of the crosslinked polymer as starting material according to example 8, but the pH was raised to 12 (as for the production of polymer

(2)) and the solution was stirred for 24 hours at 35°C.

25.2. After 6 extractions with ethyl acetate the aqueous solution was acidified to pH = 5 by the addition of Dowex 50 W X 8, filtered, re-extracted twice with ethyl acetate and freeze dried. 30 mg of an almost white powder comprising molecules (3), (6) and (7) were obtained.

25.3. The presence of free thiol groups was verified using 5,5'-dithiobis(2-nitrobenzoic acid) (*Ellman's* reagent, Sigma, Germany) in buffered aqueous medium (0.5 M phosphate buffer, pH=8), as a deep yellow color occurred.



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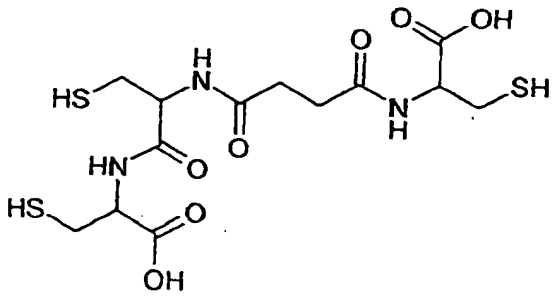

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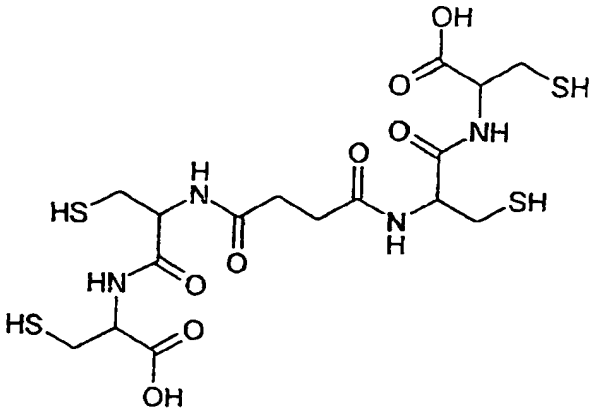

(7)

Fig. 5: Obtained molecules (3), (6) and (7) after reduction and hydrolysis

26. The mucoadhesive properties of the prepared polymers were determined according to the method established by Ch'ng et al. (J. Pharm. Sci. 74 (1985) 399-405) carried out as described in Bernkop-Schnürch et al. (Pharm. Res. 16 (6) 1999, 876-881), representing exactly the same method as described in example 1 in the instant patent application (Application No. 09/830,986).

27. The results of these mucoadhesion studies are summarized in the following table:

Tested Polymer	**Total work of adhesion (TWA) (means ±S.D.; n=3)**
Polymer 1 (according to example 1)	has no thiol groups at all
Polymer 2 (according to example 3)	has no thiol groups at all
Reduced Polymer 2 (according to example 4)	0.9 ±1.6 µJ
Crosslinked Polymer 2 (according to example 6)	has no thiol groups at all
Reduced crosslinked Polymer 2 (according to example 8)	0.7 ±1.2 µJ
Reduced crosslinked Polymer 2 with hydrolysed ester functions (according to example 9)	none [1]

[1] Test discs dissolved that rapidly in the buffer solution, that no adhesion at all could be measured.

28. None of the polymers described by Constancis displayed statistically significant mucoadhesive properties. If an inert and completely not mucoadhesive material was tested,

similar results would have been obtained. The results are in good agreement with the generally accepted theory about criteria which have to be fulfilled by a polymer in order to be mucoadhesive (e.g. G. Hunt, P. Kearney and I. Kellaway 'Mucoadhesive polymers in drug delivery systems' in Drug Delivery Systems, Ellis Horwood, New York (1987)).

29. Based upon these experiments, it can be seen that none of the polymers described by Constancis were mucoadhesive polymers. As such, it is my opinion that Constancis fails to disclose or suggest a mucoadhesive polymer to a person skilled in the art.

30. It is further my opinion that the teachings of Constancis would not have been combined with the teachings of Bernkop-Schnürch to produce the instantly claimed mucoadhesive polymers. Constancis is unrelated to mucoadhesive polymers. One skilled in the art of mucoadhesive polymers would not have looked to the bioadhesive art such as Constancis to find ways to improve mucoadhesives. The properties of the bioadhesives of Constancis are not desired in mucoadhesives.

31. I further declare that I am aware that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and my jeopardize the validity of any patent application or any patent issuing thereon. All statements made of my own knowledge are true, and all statements made on information and belief are believed to be true.

August 7, 2003
Date

Andreas Bernkop-Schnürch, Ph.D.